DOI: 10.1515/dch-2015-0002

HAPLO-IDENTICAL BONE MARROW TRANSPLANT PROTOCOL USING REDUCED INTENSITY CONDITIONING FOR FUNDENI CLINICAL INSTITUTE

Varady Zsofia^{1*}, Coriu Daniel^{1,2}, Ghiaur Gabriel³ and Richard J Jones³.

1. Bone Marrow Transplant Center, Fundeni Clinical Institute, Bucharest, Romania
2. Carol Davila University, Bucharest, Romania
3. Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Abstract:

Purpose:

Even if the romanian population is ethnically compact caucasian-type population, many of the patients referred for bone marrow transplantation lack a suitable donor. In order to expand the donor pool and the accesibility to transplant for those who have indications it is necessary to perform haplo-identical bone marrow transplant procedure in Fundeni Clinical Institute. Since 2009 Romania established a National Volunteer Stem Cell Donor Registry (RNDVCSH), the goal was to enlarge the possibility to find HLA-matched unrelated donors (MUD) for patients. This approach offered transplant for only up to 60%patients referred for transplantation in 2014, even we chose one HLA-mismatch donors.

The haploidentical transplant protocol proposed for our institution is based on Sidney Kimmel Comprehensive Cancer Center protocol from Johns Hopkins University School of Medicine. The major milestones of this protocol include: patient eligibility, donor selection criterias, evaluation of the haplo donor, the conditioning regimen plan and additional supportive care, the bone marrow harvest, prophylaxis of graft versus host disease, assessment during and after the transplant.

Donor must be HLA-haploidentical first-degree relatives of the patient with signed consent.

The patient, parents and children are typed at the allelic level for HLA-A, -B,-C,-DRB1 and -DQB1. They will perform also de HLA-antibody search using cross-match test in complement-dependent cytotoxicity.

The conditioning regimen is composed by Fludarabine 30 mg/m²/day (from day -6 to day -2) combined with Cyclophosphamide 14,5 mg/kgIBW/day (from day -6 to day-5) and TBI at 2 Gy in day -1. In case of lacking TBI procedure at 2 Gy dose in day -1, it could be replaced by two dose of Busulfan iv in day -3 and day-2 (dose=3,2 mg/kgIBW/day) for those with acute and chronic leukemias.

The donor will have general anesthesia, the target yield of marrow is 4×10^8 total nucleated cells/kg recipient using his IBW.

The GVHD prophylaxis consisted of post-transplant Cyclophosphamide (PTCy) of 50mg/kgIBW/day in IV administration in day +3 and +4, followed by tacrolimus and mycophenolatemofetil (MMF) beginning day +5. The MMF will be stopped at day+35, the tacrolimus will continue till 6 months after the transplantation.

Conclusion:

One of the most important factors affecting transplantation outcome is proper timing. Therefore, donor availability is an crucial issue. Haploidentical related donors are available for almost all patients, so the use of those donors is a viable alternative.

***Corresponding author:**

VaradyZsofia, Bone Marrow Transplant Center, Fundeni Clinical Institute, Sos. Fundeni nr.258, sector 2, Bucharest, Romania, phone:+40723293853, e-mail: zsofi2005@yahoo.

Background:

Even if the Romanian population is ethnically compact Caucasian-type population, many of the patients referred for bone marrow transplantation lack a suitable donor. In order to expand the donor pool and the accessibility to transplant procedure for those who have indications it is necessary to introduce the haplo-identical bone marrow transplant procedure with myeloablative and reduced-intensity conditioning in Fundeni Clinical Institute.

Introduction:

Bone marrow transplantation is the only curative option for many adult hematological malignancies. Donor availability in a timely manner for those patients is one of the major challenge in the treatment. The likelihood of finding an HLA-matched sibling donor (MSD) for a patient is 25% as the Mendelian rule shows, and the fact that in Romania we have more and more families with only one child, making the sibling-donor search more and more difficult. Since 2009 Romania established a National Volunteer Stem Cell Donor Registry (RNDVCSH) and the goal was to enlarge the possibility to find HLA-matched unrelated donors (MUD) for patients. This approach offered transplant for only up to 60% patients referred for transplantation in 2014, even we chose one HLA-mismatch donors. For these reasons it is important to introduce HLA-haploidentical transplantation.

Potential HLA-haploidentical donors include biological parents or children of a patient, and each sibling has a 50% chance of sharing one HLA-haplotype. So it is a rapid possibility to find a donor and it will be available in very short time for the transplant. The major drawback is the intense bidirectional alloreactivity with graft failure and graft versus host disease (GVHD), who leads to increased non-relapse mortality (NRM). Initially in haplo-setting, knowing that T-cells are responsible in graft versus host disease (GVHD), studies were made removing T cells from the donor graft ex-vivo. But the graft failure increased. The post-transplant high-dose Cyclophosphamide (PTCy) it was the next step in order to deplete in-vivo alloreactive T cells from the donor and the host at the same time, and reduce both GVHD and rejection. This DNA-damaging agent take action over the proliferative, alloreactive T cells, but not over resting T cells and allow a better immune reconstitution and lower non-relapse mortality (NRM).

Patient eligibility

Patients over 18 years of age and under 65, with a diagnosis of hematological malignancy, having an indication for allogeneic transplant within the Fundeni Clinical Institute local practice. The eligibility should be assessed by the Fundeni Clinical Institute transplant committee after the hematology specialist fulfilled the transplant-specific medical chart of the patient. The patient must have one or more potential related mismatched donor (biological parents, siblings or children) typed in high-resolution for HLA-A, -B, -C, -DRB1 and -DPB1.

The exclusion criterias are:

- a) hematological malignancy in evolution
- b) suitable matched sibling or unrelated donor, defined by local committee
- c) prior autologous stem cell transplantation, if the disease relapse under 6 months after autologous transplant.

- d) prior allogeneic stem cell transplant
- e) poor performance status: Karnofsky under 70%
- f) poor cardiac function: left ventricular ejection fraction under 45%
- g) poor pulmonary function: DLCO, FEV1 and FVC under 50%
- h) poor liver function: transaminase more than 3 time of normal level and bilirubin over 2,5 mg/dl (except the Gilbert syndrome)
- i) poor renal function: elevated creatinine above 1,5 mg/dl.
- j) current uncontrolled bacterial, viral or fungal infection
- k) anti-donor HLA-antibodies defined by positive crossmatch test of any titer rely on complement-dependent cytotoxicity
- l) evidence of HIV positive serology or infection
- l) pregnancy and breast-feeding.
- m) lack of a signed informed consent for the patient and for his donor.

After the patient passed this exclusion criterias he will be screened for HbsAg; anti Hbcore; anti HIV 1,2; HIV1,2 NAT; HCV NAT; HBV NAT; anti-HTLV; anti-HCV; anti-CMV, anti-EBV; anti-toxoplasma and serology for syphilis.

Donor selection criterias, in decreasing order of priority:

1. donor must be medically, socially and psychologically fit to donate
2. donor must be HLA-haploidentical first-degree relatives of the patient with signed consent. For donors under 18 years of age a local judge will take the consent, obeying Romanian rules and laws.
3. the maximum recipient actual body weight should not exceed 1,25 times the donor actual body weight.
4. the patient must lack antibodies against donor HLA molecules (specifically complement dependent cytotoxicity)
5. no major ABO incompatibility between donor and recipient (include: recipient "O" with donor "A", "B" or "AB"; recipient "A" with donor "B" or "AB"; recipient "B" with donor "A" or "AB")
6. matched CMV IgG serologic status between donor and recipient (for a recipient who is CMV negative, use a CMV negative donor; for a positive recipient use a positive donor)
7. use an ABO compatible donor over a minor ABO incompatible donor.
8. other donor characteristics (in no order of priority): preferring donors above 18 years of age over donor under 18; among donors above 18, prefer younger and lighter donor; for male recipients male donors are preferred.

Evaluation of the haplo-identical donor

Haplo-identical bone marrow donor has to be extensively evaluated before donation in order to assess the potential risks to the donor and recipient. Donor eligibility must be documented prior to the start of the preparative regimen for the recipient in the recipient chart. This task will be completed by the transplant physician who will take care of the recipient, under the supervision of Fundeni Clinical Institute transplant committee.

The donor identification must fulfill the following steps:

1. After a patient (recipient) referral to allogeneic transplant setting, HLA typing of the patient and appropriate family members is conducted in an EFI accredited facility using high-resolution technique. The patient, parents and children are typed at the allelic

level for HLA-A, -B, -C, -DRB1 and -DQB1. They will perform also de HLA-antibody search using cross-match test in complement-dependent cytotoxicity. Based on the HLA results and cross-match, potential donors are identified. The laboratory will compose a histocompatibility report and they will submitted this report to Fundeni Clinical Institute transplant committee and it will be attached to the patient medical chart by the transplant physician.

2. When more than one donor is identified, they all are screened using medical history questionnaire, physical examination and laboratory investigations supervised by the transplant physician. The laboratory investigations will be all of the following: CMV status, ABO and Rhesus blood group. He will also generate a report submitted to the Fundeni Clinical Institute transplant committee and attached to the patient medical chart.
3. The transplant committee will discuss and choose the appropriate donor upon the HLA report and clinical status report mentioned at previous two paragraphs. The committee's choice will be attached to the patient medical chart by the transplant physician.
4. The choosed donor than has to be investigated furthermore by the transplant physician as follows:

a) verification of HLA typing in a second set of blood-drawn performed for the patient and the identified donor

b) the donor will be screened for relevant communicable diseases obeying local policy for: HbsAg; anti Hbcore; anti HIV 1,2; HIV1,2 NAT; HCV NAT; HBV NAT; anti-HTLV; anti-HCV; anti-CMV, anti-EBV; anti-toxoplasma and serology for syphilis.

c) the donor will have an EKG, pulmonary radiology and if she is female donor: a pregnancy test.

d) the donor will have an anesthesia evaluation at the Anesthesia Department of Fundeni Clinical Institute.

All the documentation mentioned above at point 4 will be done by the transplant physician and kept in the patient medical chart.

The conditioning regimen plan and the additional supportive care:

The treatment plan for the haplo-identical transplant setting it is a non-myeloablative conditioning regimen, using the following:

<i>Day</i>	<i>Drug</i>	<i>Dose</i>	<i>Mode of administration</i>
-6	Fludarabine Cyclophosphamide Uromitexan	30 mg/m ² 14,5 mg/kg IBW 17,4 mg/kg IBW	IV over 60 minutes IV over 2 hours IV continuous infusion for 24 hours
-5	Fludarabine Cyclophosphamide Uromitexan	30 mg/m ² 14,5 mg/kg IBW 17,4 mg/kg IBW	IV over 60 minutes IV over 2 hours IV continuous infusion for 24 hours
-4	Fludarabine	30 mg/m ²	IV over 60 minutes
-3	Fludarabine	30 mg/m ²	IV over 60 minutes
-2	Fludarabine	30 mg/m ²	IV over 60 minutes
-1	TBI	2 Gy in a single fraction	Local policy

0	Infusion of bone marrow		Local policy
3	Cyclophosphamide Uromitexan	50 mg/kg IBW 60 mg/kg IBW	IV over 2 hours IV continuous infusion over 24 hours
4	Cyclophosphamide Uromitexan	50 mg/kg IBW 60 mg/kg IBW	IV over 2 hours IV continuous infusion over 24 hours
5	Tacrolimus Mycophenolatemofetil GCSF	0,12 mg/kg/day 15 mg/kg/dose 5 mcg/kg/day	PO daily divided in 2 doses PO divided in 2-3 doses IV or SC

In case of lacking TBI procedure at 2 Gy dose in day -1, it could be replaced by two dose of Busulfan iv in day -3 and day-2 (dose=3,2 mg/kgIBW/day) for those with acute and chronic leukemias.

The IBW (ideal body weight) will be calculated using Devine formula and the clearance of creatinine will be calculated using the Cockcroft formula. The fludarabine will be reduced with 20% if de creatinine clearance is below 70 ml/min.

The following additional supportive measures will be kept within this protocol:

1. hydration, anti-nausea treatment, fluid-balance, transfusional and nutritional support will respect Fundeni Clinical Institute local standards
2. the central venous access and his care will respect institutional practice.
3. it will be minimum 4-6 hours between the TBI and infusion of bone marrow cells
4. if there is a major ABO incompatibility the marrow will be red-cell depleted using institutional practice
5. prophylaxis for antibacterial, antifungal and antiviral agents will be start in day 0 using institutional practice. The stop of this drugs will respect also the local policy.
6. post-transplant cyclophosphamide will start at minimum 60-72 hours after the start of marrow infusion
7. it is prohibited to use corticosteroids until at least 24 hours after the finish of cyclophosphamide infusion. The only exception is using corticosteroids if anaphylaxis.
8. residual serum level of tacrolimus will be first measured at day +7 and it will be kept between 5-15 ng/ml using institutional practice till 6 months after transplant.
9. every tacrolimus dose change will be controlled by residual serum level at 48 hours
10. mycophenolatemofetil prophylaxis will be ended at day +35.
11. GCSF will be given until absolute neutrophil count is above 500/mm³ for three consecutive days.
12. thetacrolimus levels and PCR-CMV will determined every week within the first 60 days and wherever is clinically indicated.

Bone marrow harvest

The bone marrow harvest will respect the Fundeni Institution local practice. It will be

performed in operating theater by the one hematologist or transplant physician and one 3-5 year hematology resident with the help of two nurses from the Fundeni Stem Cell Bank. The donor will have general anesthesia done and supervised by the ICU physician. The target yield of marrow is 4×10^8 total nucleated cells/kg recipient using his IBW. Minimum recommended yield will be $2,5 \times 10^8$ total nucleated cells/kg recipient using his IBW. A sample of the product to be infused will be sent to flow-cytometry to determine the CD34+ cell count.

If the marrow has major ABO incompatibility it will be depleted in red cells using institutional standards. The marrow will be infused in the same day to the patient.

Graft versus host disease (GVHD) prophylaxis

The GVHD prophylaxis consisted of post-transplant Cyclophosphamide (PTCy) of 50mg/kgIBW/day in IV administration in day +3 and +4, followed by tacrolimus and mycophenolatemofetil (MMF) beginning day +5. The tacrolimus and MMF will be used in oral administration as the transplant protocol show above. The MMF will be stopped at day+35, the tacrolimus will continue till 6 months after the transplantation. If GVHD occur it will be addressed using Fundeni institutional policies.

Risks and toxicities

1. Cyclophosphamide side effects could be : nausea/vomiting, cardiomyopathy, skin rash, mucositis, sterility, hemorrhagic cystitis, fluid gain and edema with weight gain, hemolysis and alopecia.
2. Fludarabine side effects could be : neurotoxicity (agitation, confusion, blurred vision, peripheral neuropathy, loss of hearing, weakness, blindness, coma), autoimmune hemolytic anemia, deep venous thrombosis, transient ischemic attack, phlebitis, fever, chills, skin rash, nausea/vomiting, diarrhea, stomatitis, anorexia, abnormal liver function tests, abnormal renal function tests, peripheral edema, myalgia, pulmonary toxicity.
3. Total Body Irradiation can cause : nausea/vomiting, parotiditis, diarrhea, erythema, fever, mucositis, alopecia. Late effects include hyperpigmentation, cataract, risk for secondary malignancies, sterility, pneumonitis, nephropathy.
4. Tacrolimus side effects could be: renal insufficiency, hypertension, hypomagnesemia, hypokalemia, hyperglycemia and neurologic toxicity (tremor).
5. Mycophenolatemofetil side effects could be: nausea/vomiting, diarrhea, headache, hypertension, dizziness, pancytopenia, insomnia, rash, bone pain, electrolyte imbalances, hyperglycemia.

Assessment during the transplant

During the transplant (and in neutrophil-recovery period) patient will be hospitalized in Bone Marrow Transplant Unit at Fundeni Clinical Institute respecting the local institutional practice for hospitalized patients. He will be in care of a transplant physician. Before admittance all patients will have a bacterial portage evaluation (nasal and pharyngeal swabs, cultures of urine and stool).

The transplant phase will be assessed as follows:

1. daily physical examination for the assessment of general health and well-being, infectious complications, medication related problems and GVHD.
2. Daily CBC (complete blood count).
3. At least three times weekly (or wherever needed): creatinine, uric acid, bilirubin, LDH, AST, FAS, Na, K, Cl, Ca, Mg, Ph, blood sugar.

4. Weekly (or wherever needed): coagulation panel, residual tacrolimus level, PCR-CMV.
5. Cultures wherever the clinical situation of the patient indicates, using institutional practice.
6. Any other evaluations or imaging could be performed at any time with medical indication from the transplant physician who has the patient in charge.

Assessment in the post-transplant period

In the post-transplant period the transplant physician will assess the patient regularly in outpatient manner and the patient will be admitted as needed.

This phase will be assessed as follows:

1. At day +30, +60, +120, +180 and 1 year: the patient will have a complete physical examination, CBC, creatinine, uric acid, bilirubin, LDH, AST, FAS, Na, K, Cl, Ca, Mg, Ph, blood sugar, coagulation panel, residual tacrolimus level (if it needed), PCR-CMV, peripheral blood chimerism (unsorted and T cell).
2. Between 6 months and 1 year the patient will come monthly to be evaluated with: complete physical examination, CBC, creatinine, uric acid, bilirubin, LDH, AST, FAS, Na, K, Cl, Ca, Mg, Ph, blood sugar, coagulation panel, residual tacrolimus level (if it needed).
3. At day +30, +180 and at 1 year the patient will have bone marrow aspirate/biopsy, endocrinological testing and consult..Disease status evaluation with specific leukemia and lymphoma panel.
4. In the second year the patient will be evaluated at every third months with: complete physical examination, CBC, creatinine, uric acid, bilirubin, LDH, AST, FAS, Na, K, Cl, Ca, Mg, Ph, blood sugar, coagulation panel, residual tacrolimus level (if it needed).
5. In the third year the patient will be evaluated at every six months with: complete physical examination, CBC, creatinine, uric acid, bilirubin, LDH, AST, FAS, Na, K, Cl, Ca, Mg, Ph, blood sugar, coagulation panel.
6. Starting from the second year the patient will have yearly peripheral blood chimerism, disease status evaluations, endocrinological evaluations with hormone-dosage and thyroid ultrasonography, mammography and genital evaluation with Papanicolau smear for women. Cardiological (EKG and ultrasound) and pulmonary evaluation (function tests).
7. Any other evaluations or imaging could be performed at any time with medical indication.
8. The patient will be re-immunize starting from 1 year (if he is not having extended chronic GVHD) using institutional standard.

One of the most important factors affecting transplantation outcome is proper timing. Therefore, donor availability is an crucial issue. Haploidentical related donors are available for almost all patients, so the use of those donors is a viable alternative.

Acknowledgements to my host mentors Gabriel Ghiaur and Richard J Jones from the Bone Marrow Transplant Program at the Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA and the Visitor Training Program within American Society of Hematology.